INVESTIGATIONS ON RUBBER-BEARING PLANTS

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IV. CYTOGENETIC STUDIES IN *ASCLEPIAS* (TOURN.) L.[1]

By Raymond J. Moore[2]

Abstract

The chromosome number of eight species and varieties of *Asclepias* (*A. syriaca*, *A. incarnata* and variety *pulchra* and forma *albiflora*, *A. Sullivantii*, *A. tuberosa*, *A. curassavica*, *A. speciosa*) was found to be $2n = 22$.

Various interspecific pollinations were attempted without success. A cross of *A. incarnata* with forma *albiflora* was successful.

Possible natural hybrids between *A. syriaca* and *A. speciosa* and between *A. incarnata* and var. *pulchra* and forma *albiflora* are described. Two unusual chimaeral plants of *A. incarnata* that produced several stems of forma *albiflora* are described.

Introduction

In the course of recent wartime investigations of the possible economic uses of the genus *Asclepias*, chiefly the species *A. syriaca* L. and *A. incarnata* L., collections of these two and of several other species were assembled. As a preliminary to breeding work, the chromosome number of all the species and varieties was determined. Numerous attempts at intraspecific crosses of *A. syriaca* were made to work out a technique for artificial pollination of the curiously modified *Asclepias* flower. In addition, interspecific pollinations, involving all of the species available, were undertaken with a view to obtaining a hybrid that would surpass the native species in either latex or floss production.

Materials and Methods

Seed was collected from native plants of *A. syriaca*, of *A. incarnata*, and of *A. Sullivantii*. Seed of other species was obtained from various Botanic Gardens. Plants were grown in plots at the Dominion Arboretum and Botanic Garden, Ottawa.

Herbarium specimens from these cultures are to be found in the Herbarium of the Division of Botany and Plant Pathology, Department of Agriculture, Ottawa.

Chromosome counts were made from fresh smears of growing leaves and root tips fixed in Carnoy's mixture, made up as follows:

3 parts chloroform,
2 parts 95% ethanol,
1 part glacial acetic acid.

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The tissue was then stained in 45% aceto-orcein, heated, and flattened on the slide.

For one species, *A. curassavica*, root tips fixed in Allen's Bouin fluid, embedded, sectioned, and stained with Gentian violet were studied as well.

Cytological Observations

The diploid chromosome number of six species and two subspecific forms of one of these species was found to be 22. These counts, with previously reported determinations, are listed in Table I.

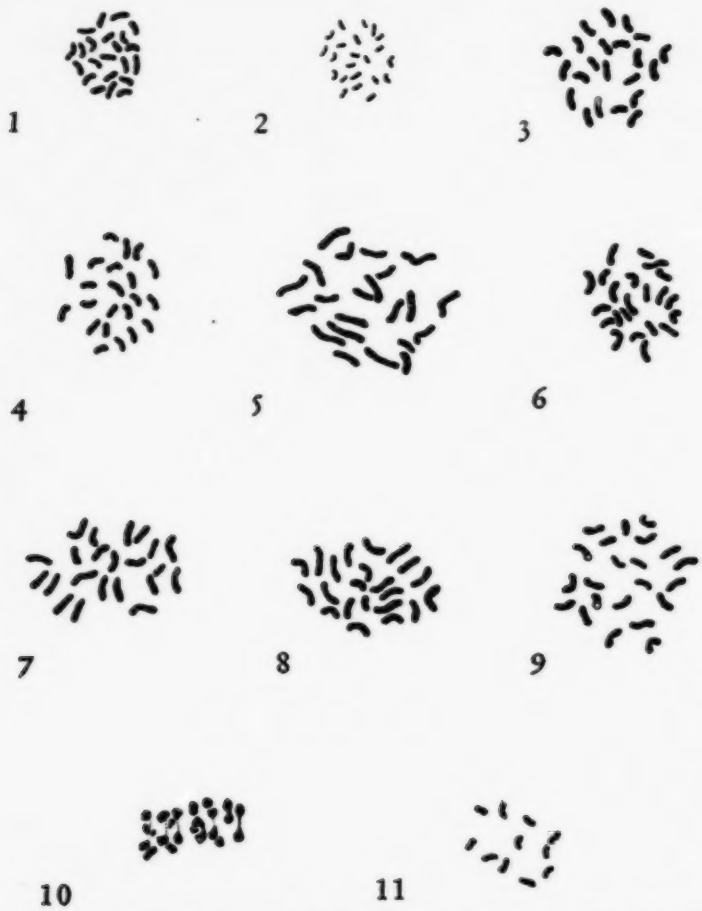
TABLE I

CHROMOSOME NUMBERS IN *Asclepias* (TOURN.) L.

Species	*n*	2*n*	Author
curassavica L.		22	Moyer, 7
curassavica L.		22	Moore
incarnata L.		22	Moyer, 7
incarnata L.		22	Moore
incarnata L. forma *albiflora* Heller		22	Moore
incarnata L. var. *pulchra* (Ehr.) Pers.		22	Moore
latifolia Raf.		22	Moyer, 7
salicifolia Lodd.		22	Moyer, 7
speciosa Torr.		22	Moore
Sullivantii Engelm.	*ca.* 5		Frye, 5
Sullivantii Engelm.		22	Moore
syriaca L.	10		Strasburger, 11
syriaca	12		Stevens, 10; Gager, 6; Finn, 2
syriaca		22	Eichhorn and Franquet, 1
syriaca L.		24	Moyer, 7
syriaca L.	11	22	Moore
tuberosa L.	5		Frye, 4
tuberosa L.		22	Moore
tuberosa L. var. *sulfurea* L.		22	Moyer, 7
verticillata	*ca.* 8		Frye, 5

The chromosomes varied in length, in the preparations used, from 1 to 2.5 μ. It is not possible to recognize with certainty any of the homologous chromosomes although two or three pairs are somewhat longer than the others. Moyer (7) remarked that there was a noticeable difference in the morphology of the chromosomes of the different species. His figures show the chromosomes of *A. curassavica* to be smaller than those of five other species (see Table I). In the present investigation it was noted that the chromosomes of this species, in both root tips and leaf smears (Figs. 1, 2) were smaller than those of the other species examined. Differences between the chromosomes of the latter species were very slight. Some variation in morphology is very likely due to the physiological condition of the tissue and the rate at which mitoses have been taking place. For this reason it is felt that further comment on this variation is not justified.

In view of the disagreement (Table I) over the chromosome number in *A. syriaca* L. (*A. cornuti* Decaisne) special attention was paid to this species. Plants grown from seed collected from eight roadside stations about Ottawa, Ont. were examined but only the diploid number of 22 was found (Fig. 10). In Fig. 11 is shown the haploid number of 11 at II meiotic metaphase while in Fig. 10 are seen the 11 bivalents of I metaphase. Several additional counts of $2n = 22$ were made by Dr. Margaret Landes, Division of Botany and Plant Pathology, Department of Agriculture.



All figures were drawn at a magnification of 2000 except Fig. 10, $\times 1700$. FIG. 2 *shows somatic chromosomes from root-tips; Figs.* 1 *and* 3 *to* 9 *show the diploid chromosomes from leaf tissue.*

1. *A. curassavica* L. 2. *A. curassavica* L. 3. *A. incarnata* L. 4. *A. incarnata* forma *albiflora* Heller. 5. *A. incarnata* variety *pulchra* (Ehr.) Pers. 6. *A. speciosa* Torr. 7. *A. Sullivantii* Engelm. 8. *A. tuberosa* L. 9. *A. syriaca* L. 10. *A. syriaca* L. I *meiotic division,* 11 *bivalents.* 11. *A. syriaca* L. II *meiotic division,* $n = 11$.

Hybridization

Technique

As is well known, the *Asclepias* flower is highly modified for insect pollination. The female parts, two separate ovaries, are almost entirely enclosed by a tube formed by the flattened filaments of the five stamens. Pollen is developed in flat club-shaped sacs (pollinia) of which there are two in each stamen. A thin tough band (retinaculum) fastens the pollinia in pairs, one member from each of two adjacent stamens. The upper portion of the styles of the two ovaries are united into a flat round stigma disk. Spaced at equal intervals around the margin of the lower surface of the disk are five stigmatic areas. Each stigmatic surface is thus located within a chamber, the outer walls of which are formed by portions of two adjacent flattened filaments, and is accessible to pollination only through a slit-like passage between the edges of the two filaments. By means of the retinacula, pollinia are readily entangled on the legs of insects gathering nectar and cross-pollination is effected by the insertion of one or more pollinia into the stigmatic chambers of the flower that the insect visits next.

In artificial pollinations, the pollinia were withdrawn from their pouches by means of a needle or fine pointed forceps and inserted into the chambers with an inward and upwards motion. Care was taken to cause as little injury as possible to the filament wings covering the entrance to the chamber. It was found after some preliminary attempts that no success was attained unless at least two adjacent chambers were pollinated with at least one pollinium each. From the arrangements of the stigmatic surfaces relative to the free styles, it appears that a passageway for pollen tubes to the same ovary will exist from four of the five possible pairs of adjacent stigmas. It was estimated that a pollinium of *A. syriaca* contains approximately 250 pollen grains. The greatest number of mature seeds obtained from a single pod was 243. It appears, therefore, that germination of a very high percentage of the pollen grains is required to fertilize all of the ovules present. Probably all of the grains in a sac do not germinate, or, if they do, cannot reach the stigmatic surface. It may be that pollen tubes from two stigmas are needed to fertilize a sufficient number of ovules to cause the ovary to remain attached to the stem.

Mature but unopened buds were used in artificial pollinations. The clusters were protected from subsequent insect pollination by paper bags. Usually only three to five flowers in an umbel were manipulated, the others were removed.

Following this technique a considerable number of intraspecific cross-pollinations of *A. syriaca* were successful.

The following interspecific hybridizations were attempted. The number of flowers crossed was relatively small. The approximate total number is indicated after each pollination. In all cases the female parent is named first. Pollinations in which some ovaries enlarged but aborted before maturity are indicated by an asterisk.

A. syriaca × *A. tuberosa* (50)
A. syriaca × *A. incarnata* (28) and *A. incarnata* f. *albiflora* (6)
A. syriaca × *A. speciosa* (10) and reciprocal (15)
A. speciosa × *A. tuberosa* (20)
A. Sullivantii × *A. syriaca* (15)
A. Sullivantii × *A. speciosa* (7)
A. Sullivantii × *A. tuberosa* (9)*
A. incarnata × *A. incarnata* v. *pulchra* (20) and reciprocal (30)
A. incarnata × *A. incarnata* f. *albiflora* (20) and reciprocal (12)
A. incarnata v. *pulchra* × *A. incarnata* f. *albiflora* (6) and reciprocal (20)
A. curassavica × *A. syriaca* (6)*
A. curassavica × *A. tuberosa* (9) and reciprocal (4)
A. curassavica × *A. incarnata* v. *pulchra* (4)
A. curassavica × *A. incarnata* f. *albiflora* (5)
A. curassavica × *A. speciosa* (4)* and reciprocal (6)*

Mature pods were obtained only in the cross *A. incarnata* × *A. incarnata* f. *albiflora*. It is felt that difficulties of manipulating the smaller flowers of *A. incarnata* were largely responsible for failure to obtain seed of the *A. incarnata* variety crosses.

After certain of the above cross-pollinations, as indicated, definite enlargement of the ovary was observed. The pod however did not mature and only small shrivelled seeds were found within. It is possible that fertilization occurred in these crosses but the seed aborted before maturity.

Seed gathered from *A. speciosa* plants in experimental plots in which *A. syriaca* also was growing produced plants that appeared to be hybrids between these species. Probably insect pollination was successful in effecting this cross. These possible hybrids showed intergradations in the leaf, flower, and stem characters of the two species.

Leaves of *A. syriaca* vary considerably in form and size—from almost round to linear-ovate and from 7 to 20 cm. in length—while those of *A. speciosa* are cordate-oval or oblong, generally smaller and covered with a silvery bloom, due to a fine pubescence. Typical *A. syriaca* plants reach 9 to 12 dm. in height, while those of *A. speciosa* are generally about 6 dm. The two species are separated most easily by their floral characters, especially the length of the hood. Hoods of *A. speciosa* are much longer (9 to 15 mm.) than those of *A. syriaca* (less than 7 mm.).

The hybrids could be classed as "*syriaca*-like *speciosa*" and as "*speciosa*-like *syriaca*". The former type resembled *A. syriaca* in regard to leaf size and shape and stem height, but the leaves bore the pubescence of *A. speciosa*. However, judging by the hood length (9 to 10 mm.), this type was to be placed in the latter species.

The "*speciosa*-like *syriaca*" plants were similarly placed within the species *syriaca* on the basis of hood size. They resembled *A. speciosa* in leaf shape and texture and in the lesser stem height.

Cross-pollinations of these possible hybrids with more typical individuals of each species were attempted; the results are shown in Table II.

TABLE II

RESULTS OF ARTIFICIAL POLLINATIONS OF TYPICAL *A. speciosa* AND *A. syriaca* WITH INTERGRADING FORMS OF BOTH SPECIES

Pollination	No. fls. pollinated	No. pods obtained
A. syriaca (*speciosa*-like) × *A. speciosa*	7	1
A. speciosa (*syriaca*-like) × *A. syriaca*	4	0
A. speciosa (*syriaca*-like) × *A. syriaca* (*speciosa*-like)	4	2
A. speciosa × *A. syriaca* (*speciosa*-like)	11	2
A. speciosa (*syriaca*-like) × *A. speciosa*	3	0

Similar supposed hybrids between these species were mentioned by Stevens (9). Moreover, this author was successful in obtaining seven mature pods from approximately 90 flowers of *A. syriaca* pollinated artificially with pollen of *A. speciosa*.

Possible hybrids of *A. incarnata* with the variety *pulchra* and the form *albiflora* were grown from seed gathered from plants in experimental plots containing these three types.

Asclepias incarnata variety *pulchra* (Ehr.) Pers. is distinguished from the species by its broader leaves and greater degree of pubescence on stem and leaf. The stem of the species is glabrous or bears only a slight line of hair. Further, variety *pulchra* has paler flowers, dull rose in colour, in contrast to the bright rose of the species.

These apparently hybrid plants showed the *pulchra* flower colour associated with the relatively glabrous stem and the leaf characters of the species. Varying degrees of stem pubescence also occurred. The association of the *incarnata* flower colour with the pubescence and leaf shape of the variety has not been seen. Seed of a single pod frequently produced a population including individuals with *pulchra* flower colour and glabrous stem and other plants completely typical of the species.

The form *albiflora* differs from the species only in pigmentation. The flowers of the former are white and pigment is wholly lacking in stems and flower peduncles and pedicels. Apparent hybrids between this form and the species also were found. Seed of a single pod of an *A. incarnata* plant sometimes produced plants that appeared typical and others that lacked pigment and were identical with the form *albiflora*.

The cross *A. incarnata* f. *albiflora* × *A. incarnata* was made artificially. All individuals (11) of the F_1 generation showed the bright rose flower colour and rose-purple stem pigmentation of *A. incarnata*. Presumably flower colour and stem pigmentation is genetically dominant to the lack of these characters.

This conclusion was further supported by the ratio between the number of white and of coloured individuals in the natural hybrid populations referred to above.

From these observations it seems probable that the three forms of this species can be hybridized and that difficulties in technique were the cause of the failure of some of the cross-pollinations attempted.

Two plants were observed that apparently were chimaeras of tissue of *A. incarnata* and of the form *albiflora*. In these, several pigmented stems bearing coloured flowers and, as well, some unpigmented stems with white flowers arose from a single root. Examinations of leaf smears from the two kinds of stems revealed that the chromosome number was the same in both, $2n = 22$. It seems therefore that the colour difference was caused by a gene mutation rather than by either a duplication or loss of a whole chromosome in either form. These plants were grown from seed collected from plots of *A. incarnata* f. *albiflora*. It is quite possible that the seed plants had been crossed by insect activity with nearby plants of *A. incarnata*. In this case it would be expected that the seed would produce heterozygous plants showing the dominant characters of the species. It is therefore impossible to say whether the mutation produced *albiflora* stems from pigmented tissue or the reverse. Only four plants survived to maturity in this plot, the other two being phenotypically *A. incarnata*. A view of the root of one of these plants, showing three unpigmented and four pigmented stems, is seen in Fig. 12.

Self-fertility

Self-pollination of *A. syriaca* and of *A. incarnata* was attempted but always without success. That self-fertile plants of both species exist has been reported by Plotnikova (8) and Stevens (9) for *A. syriaca*, and for *A. incarnata* by Fischer (3).

Acknowledgments

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PLATE I

FIG. 12. *Root and lower part of stems of a chimaeral plant of Asclepias incarnata L. showing three unpigmented and four pigmented stems.*

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